CALIFORNIA ENVIRONMENTAL PROTECTION AGENCY
DEPARTMENT OF PESTICIDE REGULATION
MEDICAL TOXICOLOGY BRANCH

SUMMARY OF TOXICOLOGY DATA

CHLORPYRIFOS

Chemical Code # 000253, Tolerance # 00342
SB 950 # 221

Summary initiated: 5/8/86
Revisions on 8/11/86, 11/24/86, 6/5/87, 4/25/89, 11/09/89, 3/16/90, 11/8/90, 5/11/92, 6/28/93, 7/19/94, 9/3/97, 11/13/98, 10/13/99, and 9/27/01

I. DATA GAP STATUS

Chronic, rat:	No data gap, possible adverse effect
Chronic, dog:	No data gap, no adverse effect
Oncogenicity, rat:	No data gap, no adverse effect
Oncogenicity, mouse:	No data gap, no adverse effect
Reproduction, rat:	No data gap, no adverse effect
Teratology, rat:	No data gap, no adverse effect
Teratology, mouse:	No data gap, no adverse effect
Gene mutation:	No data gap, no adverse effect
Chromosome effects:	No data gap, no adverse effect
DNA damage:	No data gap, possible adverse effect
Neurotoxicity:	No data gap, no adverse effect

Note, Toxicology one-liners are attached

All relevant record numbers for the above study types indexed by DPR as of 9/27/01 were examined. These include data through Document No. 342-833, Record No. 182482, plus records of the 900,000+ series. Aldous, 9/27/01.

Updated by H. Green and C. Aldous, 5/11/92; Kellner and Gee, 6/28/93 and 7/19/94, and Aldous, 9/3/97, 11/13/98, 10/13/99, and 9/27/01.

Filename: t20010927.wpd

II. TOXICOLOGY ONE-LINERS AND CONCLUSIONS

In the one-liners below:
** indicates an acceptable study.
Bold face indicates a possible adverse effect.

These pages contain summaries only. Individual worksheets may contain additional effects.

TABLE OF CONTENTS

COMBINED, RAT

** 345 072300 "Chlorpyrifos: 2-year dietary chronic toxicity-oncogenicity study in Fischer-344 rats". Dow Chemical Co., Freeport TX, 12/23/88. Chlorpyrifos ("AGR 214637"), 98.5%, in diet at 0, 0.05, 0.1, 1, and 10 mg/kg/day. 10/sex/dose designated for 1-year interim sacrifice: 50/sex/dose designated for 2-year duration. Cholinesterase (ChE) inhibition NOEL = 0.05 mg/kg/day (based on slight plasma ChE inhibition at 0.1 mg/kg/day in females). Acetylcholinesterase ChE inhibition NOAEL of 0.1 mg/kg/day is nevertheless supportable, considering the issues discussed in the review for 354:074494. The NOEL for effects other than ChE inhibition was 0.1 mg/kg/day [based on very slight ($\leq$ 3%) but often statistically significant body weight decrease in 1 mg/kg/day males]. Body weights were statistically significantly reduced in 10 mg/kg/day males (7 to 9% throughout study). The "non-ChE effects" NOAEL was 1 mg/kg/day. Findings at 10 mg/kg/day were frequent perineal yellow staining in females, approximately 50% brain ChE inhibition in males and females, a slight increase in the degree of vacuolation of the adrenal zona fasciculata (males only), and a slight increase in diffuse retinal degeneration in 10 mg/kg/day females. None of these findings indicates possible adverse health effects (see review). ACCEPTABLE**.** C. Aldous, 4/21/89, 11/9/89 (see 354:074494). NOTE: Another rat study (see Record No. 153114 under AOncogenicity, Rat@ similarly identified retinal atrophy and cataracts at the highest dose tested (100 ppm in the latter case).

342-354 074494 "Chlorpyrifos: 13-week dietary toxicity study in Fischer - 344 rats". Lake Jackson Research Center [The Dow Chemical Co.], Freeport, Texas, 12/28/88. This study was submitted by Dow to contest the CDFA decision of a cholinesterase (ChE) NOEL at 0.05 mg/kg/day in the 2-year study, 345:072300. No comprehensive CDFA review of this subchronic study is necessary at this time, since the purpose of the 13-week study was to set dose levels for the cited 2-year study, which has already been accepted by CDFA. This subchronic study found statistically reduced plasma ChE levels ($p < 0.05$, two tailed) at day 44, but not at day 91. Investigators concluded findings at day 44 "not considered to be of toxicologic or biologic significance." CDFA concludes that the findings are probably treatment effects, which however have no apparent toxicological consequence: the plasma ChE NOEL remains 0.05 mg/kg/day, but a practical NOAEL for ChE inhibition is 0.1 mg/kg/day. C. Aldous, 11/9/89.

342-363 087917 (supplemental information to 342-345:072300). "Macroscopic postmortem examination of the eyes and associated structures in albino rats (Dow Method)". (Refers to technique used at Freeport, TX, facility), method description dated 9/11/89. Methodology was presented in accordance with a CDFA request, which was made in the 4/21/89 CDFA review of the cited study. C. Aldous, 3/16/90.

250-251 036335-036337 "Results of Two-Year Dietary Feeding Studies on DOWCO 179 in Rats" Dow Chemical, Midland, Michigan, 9/20/71. Chlorpyrifos, (presumed technical); 0, 0.01, 0.03, 0.1, 1.0, and 3.0 mg/kg/day in diet. NOEL cholinesterase enzyme inhibition = 0.1 mg/kg/day. NOEL for

other systemic effects = 3.0 mg/kg/day (HDT). No oncogenicity observed. Incomplete, UNACCEPTABLE, and not upgradeable Too few animals, too much attrition due to disease (largely chronic murine pneumonia) & dose levels not justified and apparently below the MTD. C. Aldous, 1/28/86.

EPA 1-liner: [2-year feeding, rat, Dow Chemical Co, 9/20/71] Systemic NOEL 3.0 mg/kg/day (HDT); ChE NOEL = 0.1 mg/kg/day. Carcinogenic potential negative up to 3.0 mg/kg/day (HDT). Core grade, Supplementary.

044 031074 Published summary of 250/251:036335-036337.

013/053 031070 Summary of 250/251:036335-036337.

CHRONIC RAT

(See combined rat, above)

CHRONIC DOG

**252 036338-036339 "Results of Two-Year Dietary Feeding Studies on DOWCO* 179 in Beagle Dogs," Dow Chemical, Midland, MI, 12/10/71. Chlorpyrifos, (97.2% by glc); 0, 0.33, 1.0, 3.3, 33, and 100 ppm in feed. Cholinesterase inhibition NOEL = 1.0 ppm. NOEL for other effects, including behavioral observations, was the HDT = 100 ppm. ACCEPTABLE, no adverse effects: upgraded 3/16/90 on receipt of details on preparation of treated food. (Previous objections of CDFA to this study were (1) concerns that dosage range may not have adequately challenged the dogs, and (2) lack of reporting of ophthalmological examination data in the final report. These were addressed in submissions 306:063996 and 338:070883, respectively.) C. Aldous, 1/29/86, 4/11/89, and 3/16/90 (see also rebuttal response of 6/4/87 and minutes of meeting with Dow Chemical Co. representatives on 6/29/88).

342-363 087918 (Addendum to 342-252:036338, combined dog study). Submission contains mean body weights/sex and average food consumption for a 6-week period. At the end of the 6-week period, it was determined that 100 ppm in diet corresponded closely to 3.0 mg/kg/day in either sex. From that time on, diets were prepared at fixed levels of 100, 33, 3.3, 1.0, and 0.33 ppm by serial dilutions of diets. These data permit an upgrade of the 1971 dog study to ACCEPTABLE status. Aldous, 3/16/90.

273 056902 (Tab 3) EPA Office of Pesticide Programs, Toxicology Branch review of study 252:036338-036339. The review was submitted on Oct. 10, 1985 as OPP Toxicology Branch Document #004712. The review classified the study as "Core Minimum Data".

EPA 1-liner: [2-year feeding - dog; Dow Chem. Co.; 12/10/71] Systemic NOEL = > 3.0 mg/kg/day (HDT); Plasma ChE NOEL = 0.01 mg/kg/day; Plasma ChE LEL = 0.10 mg/kg; RBC ChE NOEL = 0.10 mg/kg/day; RBC ChE LEL = 1.0 mg/kg; Brain ChE NOEL = 1.0 mg/kg/day; Brain ChE LEL = 3 mg/kg; Core grade, supplementary [**NOTE UPGRADE TO "CORE MINIMUM" STATUS, INDICATED IN 273:042783**].

306:063996 "Results of 93-day dietary feeding studies of O,O-diethyl O-3,5,6-trichloro-2-pyridyl phosphorothioate in beagle hounds". This study was evaluated with respect to study 252:036338 in the 4/11/89 CDFA review.

338:070881-070882 are dietary analyses and analytical methods descriptions. These data were evaluated with respect to study 252:036338 in the 4/11/89 CDFA review.

338:070883 is a supplement to the original 2-year dog feeding study report. Supplement included ophthalmology data. These data had been submitted to EPA in 1985. These data were evaluated with respect to study 252:036338 in the 4/11/89 CDFA review.

044 031073 Published summary of 252:036338.

013/053 031070 Summaries of 252:036338-36339.

ONCOGENICITY, RAT
(See also COMBINED, RAT section above)

****342-692 153114** Crown, S., APyrinex technical oncogenicity study in the rat@, Life Science Research Israel, Ltd., July 12, 1990. Laboratory Study # MAK/095/PYR. Pyrinex (chlorpyrifos), 96.1% purity, was administered in diet to 60 F344 rats/sex/group at 0.2, 5, and 100 ppm. There were two control groups (with and without corn oil mixing supplement), each composed of 60/sex/group. Treatment was for 2 yr, except that 5/sex/group were sacrificed at wk 50 for brain cholinesterase (ChE) assays. ChE enzyme inhibition NOEL = 0.2 ppm (inhibition of plasma ChE at 5 ppm). NOEL for non-ChE-related changes = 5 ppm. No definitive cholinergic signs were evident at any dose level. Findings at 100 ppm included modest body weight decrements and over 50% brain ChE inhibition in both sexes, and an increase over baseline incidences of diffuse retinal atrophy and cataracts in 100 ppm females. The latter findings are A**possible adverse effects**@ in an **acceptable** oncogenicity study. Aldous, 8/28/97.

ONCOGENICITY, MOUSE

**342-693 153115 Gur, E., APyrinex technical oncogenicity study in the mouse@, Life Science Research Israel, Ltd.,10/15/92. Laboratory Study # MAK/106/PYR. Fifty-nine CD-1 mice/sex/group

were dosed for 79 weeks with Pyrinex technical (chlorpyrifos) in diet at 0, 5, 50, or 250 ppm. An additional 5/sex/group were killed at week 42 for cholinesterase (ChE) evaluation. There was no ChE NOEL in the tested dosage range (dose-related inhibition of plasma ChE in both sexes at weeks 42 and 78). Brain ChE was modestly reduced at 50 ppm and greatly reduced at 250 ppm (residual activity about 20% or less in both sexes and both sampling intervals). RBC ChE was reduced at 250 ppm only. There were no definitive cholinergic signs at any dose. NOEL for other effects was 5 ppm (males displayed excessive lacrimation, opaque eyes, and hair loss around eyes: all plausibly related to contact irritability of test article with resultant scratching). High dose findings, in addition to signs consistent with local irritation, included hepatocyte vacuolation and cystic dilatation of bulbourethral glands (males), and alveolar macrophage accumulation in lungs (females). Male body weights and food consumption were decreased at 250 ppm, and water consumption was sharply reduced in both sexes at that dose level. Survival of high dose males was remarkably higher than other groups. This is an **acceptable** oncogenicity study with no adverse chronic effects. Aldous, 8/22/97.

**253 036340 "Results of a two-year toxicity and oncogenic study of Chlorpyrifos administered to CD-1 mice in the diet", Dow Chemical Toxicology Laboratory, Indianapolis, Indiana, 3/4/80. Chlorpyrifos, Ref. No. 1-500-2: 99.6% purity at 0, 0.5, 5.0, and 15.0 ppm in diet. NOEL = 15 ppm (no toxicity). No oncogenicity. ACCEPTABLE, based on re-reading of blood smears by S. D. Warner, D.V.M., Ph.D. (data in CDFA record 315:065762) answering a question by CDFA regarding possible effects on lymphocytes, (see 5/29/87 CDFA review). (Other concerns which CDFA had on this report were addressed in the 5/29/87 CDFA review). C. Aldous, 1/31/86, 5/29/87, 4/12/89.

273 042782 (Tab #4) Supplemental to 253:36340. "Chlorpyrifos: A Four -Week Dietary Study in CD-1 Mice," Dow Chemical, Midland, MI. Dietary administration of 0 or 15 ppm chlorpyrifos (95.7% purity) to CD-1 mice. 4 week study with body weights slightly reduced and plasma and serum ChE levels statistically significantly reduced (see especially. Table 13). Meets minimal requirements for an "MTD" for dose level selection for an oncogenicity study (such as 253:036340, above). Examined 11/24/86 by C. Aldous. No written review required or performed.

EPA 1-liner: [2-Year oncogenic - mice; Dow Chemical Co.; 3/04/80]: Systemic and oncogenic NOEL > 15 ppm (HDT). Core grade, minimum.

290:050623 (Rebuttal/Additional data to 253:36340) "Results of a Two-Year Toxicity and Oncogenic Study of Chlorpyrifos Administered to CD-1 Mice in the Diet". Dow Chemical Toxicology Laboratory, 3/4/80. New information consists of individual data for blood smear exams, clinical observation and animal disposition, and gross and histopathology. Reviewer (Aldous) examined previously submitted chemical analyses of test material used in this and in one other study, and included evaluation in 5/29/87 review. No adverse effects noted. Study not acceptable, but possibly upgradeable. C. Aldous, 5/29/87.

013/053 031071 Summary only of 253:036340.

REPRODUCTION, RAT

**342-399 097570 "Chlorpyrifos: Two-generation dietary reproduction study in Sprague-Dawley rats", (W. J. Breslin, A. B. Liberacki, D. A. Dittenber, K. A. Brzak, and J. F. Quast). The Toxicology Research Laboratory, Health and Environmental Sciences, The Dow Chemical Company, Midland, MI., Study ID: K-044793-088, 6/5/91). Chlorpyrifos, (technical grade Dursban F insecticide, AGR 273801), 98.5% purity, was fed in the diet to 30 Sprague-Dawley rats/sex/group through 2 generations with 1 litter per generation. Concentrations were adjusted as needed to achieve exposures of 0, 0.1, 1.0, and 5.0 mg/kg/day. Treatment began approximately 10 and 12 weeks prior to breeding for the F0 and F1 adults, respectively. Cholinesterase (ChE) inhibition NOEL = 0.1 mg/kg/day (Plasma and RBC ChE inhibition at 1.0 and 5.0 mg/kg/day). Parental NOEL = 1.0 mg/kg/day (increased degree of vacuolation in zona fasciculata, especially in males; altered tinctorial properties in this tissue in females). Reproductive NOEL = 1.0 mg/kg/day (slightly reduced pup weights and slightly reduced pup survival at 5.0 mg/kg/day). There were no clinical signs specifically indicating ChE inhibition. The reproductive findings at 5 mg/kg/day do <u>not</u> warrant a "possible adverse effects" designation, since brain ChE levels were very markedly depressed at that dose level, and all observed reproductive effects appeared to be due to failure of dams to nurture pups which were otherwise normal. ACCEPTABLE. (Green and Aldous, 5/11/92).

342-685 152365 Exact duplicate of 342-399 097570.

342-374 090493 Interim report for Record No. 097570, above.

342-686 152368 Breslin, W. J., A. B. Liberacki, D. A. Dittenber, and J. F. Quast. AEvaluation of the developmental and reproductive toxicity of chlorpyrifos in the rat@. *Fundam. Appl. Toxicol.* **29**:119-130 (1996). This is a published summary of major findings of two accepted studies: the reproduction study above (342-399 097570) and the rat teratology study (342-254 036344). Since the abstract was consistent with DPR 1-liner conclusions for the two studies, this publication was not independently reviewed. Aldous, 7/31/97.

254 036341 "Three Generation Reproduction and Teratology Study in the Rat Following Prolonged Dietary Exposure to Dursban, O,O-Diethyl O-3,5,6-Trichloro-2-Pyridyl Phosphorothioate," Dow Chemical, Zionsville, Indiana, 8/20/71. Chlorpyrifos, purity and grade not specified. Doses for the main portion of the reproduction study were 0, 0.1, 0.3, and 1.0 mg/kg/day in diet. ChE inhibition NOEL= 0.3 mg/kg/day. General adult toxicity NOEL = 1.0 mg/kg/day (HDT). Reproductive NOEL = 0.3 mg/kg/day (slightly increased pup mortality in first 5 days post-partum) <u>UNACCEPTABLE, incomplete, not upgradeable</u> (more definitive follow-up study is 254:036343). C. Aldous, 1/31/86.

(An additional copy of 036341 is found in Document No. 342-685, Tab 49 (no record #).

EPA 1-liner: [3-Generation reproduction/teratology - rat; Dow Chem. Co.; 8/20/71] Reproduction NOEL>1.0 mg/kg/day (HDT); Teratogenic NOEL = inconclusive. ChE NOEL=0.1 mg/kg Core grade, minimum

254 036343 Dietz, F. K., D. C. Mensik, C. A. Hinze, B. L., Rachunek, and H. W. Taylor, "Dursban Insecticide: Assessment of Neonatal Survival In A Two-Generation Reproduction Study In Rats," Dow Chemical, Freeport, Texas, 7/83. Chlorpyrifos, technical; 0, 0.5, 0.8, and 1.2 mg/kg/day (dietary). Parental toxicity NOEL = reproductive toxicity NOEL = highest dose tested = 1.2 mg/kg/day. UNACCEPTABLE, incomplete, upgradeability unlikely (highest dose level not demonstrably toxic, and no justification offered for dosage selection). C. Aldous 2/7/86.

EPA 1-liner: [Two generation repro - rat; Dow Chem.: 7/83] Reproductive NOEL > 1.2 mg/kg/day (HDT); Systemic NOEL = 0.8 mg/kg; Systemic LEL= 1.2 mg/kg (decreased weight gain); Core grade, supplementary.

342-681 152366 Exact duplicate of 254 036343, above.

291: [No Record #, Tab = "Reproduction"] Rebuttal comments ref. rat reproduction studies 254:036341 and 254:036343. Registrant noted that CDFA should consider both reproduction studies together, considering additionally rat chronic data. Registrant suggested that plasma and RBC ChE inhibition data support adequacy of dose. CDFA response: Doses are not justified in terms of parental toxicity, notwithstanding enzyme inhibition effects. Chronic studies are imperfect surrogate studies for evaluation of microscopic changes due to test article, since in chronic studies there is no evaluation of effects which carry over the generations. No change in status of studies. C. Aldous, 6/2/87.

342-686 152367 James, P., A. Stubbs, C. A. Parker, J. M. Offer, A. Anderson, AThe effect of Pyrinex (chlorpyrifos) on reproductive function of two generations in the rat@, Huntingdon Research Centre, Ltd., 4/22/88. HRC Report # MBS 29/881452. Crl:CD**7**(SD)BR rats received diets containing 0, 2, 10, or 50 ppm chlorpyrifos (95% purity) in diets over 2 generations (1 litter per generation). Parental rats numbered 28/sex/group in the F0 generation, and 24/sex/group in the F1 generation. Protocol was that of a standard reproduction study, with a few pre-weaning developmental evaluations added (surface righting, air righting, and startle responses; and pupil reflex). There were **no definitive treatment-related effects** (report attributes 3 high dose deaths to treatment, however there were deaths in other groups and no evident unique symptoms in high dose decedents). Study is **not acceptable** as presented (report evidently contains 401 pages, but only pp. 1-228 are present, Aconfidentiality@ stamps cover much of the text, more definitive high dose justification would be needed, and histopathology of parental rats is needed if this study is to be upgraded). Aldous, 8/22/97.

TERATOGENICITY, RAT

**342-254 036344 Ouellette, J. H., D. A. Dittenber, P. M. Kloes, and J. A. John, "Chlorpyrifos: Oral Teratology Study in Fischer 344 Rats," Toxicology Research Lab., Dow Chemical USA, Midland, MI, 7/5/83. Chlorpyrifos, 96.6%. 0, 0.1, 3.0, and 15 mg/kg/day (gavage). Maternal NOEL (excluding cholinesterase (ChE) inhibition) = 3.0 mg/kg/day (cholinergic effects). Maternal ChE inhibition NOEL = 0.1 mg/kg/day (inhibition of plasma and RBC ChE). Developmental toxicity NOEL = 15 mg/kg/day (HDT). ACCEPTABLE due to submission of supplementary information. See CDFA Rebuttal comments, C. Aldous, 6/1/87. (Study had been classified unacceptable in previous review by C. Aldous 2-10-86). C. Aldous, 6/1/87.

EPA 1-liner: [Teratology - rat; Toxicology. Research Lab; 7/5/83] Teratogenic and fetotoxic NOEL> 15 mg/kg/day (HDT); Maternal NOEL= 0.1 mg/kg; Maternal LEL= 3.0 (ChE inhibition) Core grade, minimum.

342-683 152360 (exact duplicate of 342-254 036344, above).

291 050624 (Rebuttal to primary study 254:036344). Considered in 6/1/87 review of primary study, 254:036344, above.

291 050625 (Pilot study to primary study 254:036344). "Chlorpyrifos: Oral teratology probe study in rats". Toxicology Research Lab, Dow, 1/4/83.

Chlorpyrifos, 96.6%. 0, 3, 10, and 30 mg/kg/day by gavage in cottonseed oil. Study demonstrates that 30 mg/kg/day is severely toxic to dams: maternal deaths, typical cholinergic signs, high number of resorptions. Slightly matted haircoat and slight enlargement of adrenals were observed at 15 mg/kg/day. This pilot study clearly substantiates the adequacy of the dosage range selected for the primary study, 254:036344. C. Aldous, 6/1/87.

342-695 153117 Rubin, Y., N. Gal, T. Waner, and A. Nyska, "Pyrinex teratogenicity study in the rat", Makhteshim-Agan of North America Inc., 7/15/87. Laboratory Study #MAK/101/PYR. At least 21 pregnant CD rats/group were dosed with Pyrinex Technical (chlorpyrifos), purity 96.1% by gavage in corn oil on days 6-15 p.c. at 0, 0.5, 2.5, or 15 mg/kg/day. No maternal ChE NOEL was identified (dose-related plasma ChE inhibition at all dose levels at day 15 p.c., with restoration of normal ChE activity in all but high dose dams by p.c. day 20. Maternal functional NOEL = 2.5 mg/kg/day (tremors in 3/21 dams, transient food consumption reduction, modest but consistent body weight decrement). Developmental NOEL = 2.5 mg/kg/day (slight increase in early resorptions). **No adverse reproductive effect at dose levels sufficient to elicit cholinergic responses. Acceptable. Aldous; May 1, 1997.

342-683 152361 Exact duplicate of 342-695 153117, above.

342-681 152354 Muto, M. A., F. Lobelle, J. H. Bidanset, and J. N. D. Wurpel, "Embryotoxicity and neurotoxicity in rats associated with prenatal exposure to Dursban", *Veterinary and Human Toxicology* 34, 498-501 (1992). Investigators from the Department of Pharmaceutical Sciences, St.

John=s University, Jamaica, NY. Test article was a formulation of 1% chlorpyrifos, 6% xylene, and 93% water. Suspensions were diluted to an unspecified dosing volume with saline. Dosing was ip, either on days 0-7 or on days 7-21 at dose levels of 0, 0.03, 0.1, or 0.3 mg/kg/day of chlorpyrifos. In most cases, there were 8 pregnant rats (strain unspecified) per dose for each treatment time period. Dams were allowed to litter, then pups were evaluated for Ageneral viability, body weight and physical characteristics@. Selected pups were evaluated for Aneurotoxicity@ on a rotorod on day 16. The same day, pups were evaluated for motor behavior (subjective open field observation) and for righting behavior on an inclined screen. An additional study evaluated the neurotoxicity and behavioral tests following exposures of 0.1 or 0.3 mg (presumably ip) as single doses on day 3, 10, or 12 postpartum, or as multiple doses on days 6-10 postpartum. Investigators claimed that treatment caused increased embryolethality following dosing on gestation days 0-7 and gestation days 7-21. Since the highest embryolethality was in the lowest dose group treated on gestation days 0-7 (77% lethality), these data are of questionable value. Incidences of Aphysical abnormalities@ were reportedly highest in 0.1 and 0.3 mg/kg/day groups (66 and 55%, respectively), among litters treated on gestation days 0-7. No corresponding control data were presented. Rotorod performance was reported to be impaired in pups dosed at 0.3 mg/kg on days 3, 10, and 12, and in offspring of dams dosed with 0.3 mg/kg on days 7-21, and in offspring of dams dosed with 0.03, 0.1, or 0.3 mg/kg on days 0-7. These data are suspect because differences between mean values at any treatment time dwarfed differences between dose groups at individual treatment times, even though all pups were evaluated at day 16. The study is unacceptable (in addition to deficiencies noted above, test article does not represent either the a.i. or any end use product; the route (ip) is not a plausible route of human exposure; the conclusions are speculative, evidenced by discussion of possible delayed distal neuropathy, while ignoring a valid 1986 subchronic hen neurotoxicity study, which would have been available through Afreedom of information@ provisions long before the time of this publication; and the presentation of the article shows that it could not have gone through a meaningful review, indicated by the above deficiencies, and by misspellings (the term Aaccess@ when Aassess@ was meant) and by failures to provide control data in figures or to provide numerical counts for types of purported treatment-caused malformations. No more information is requested of this paper. Aldous, 9/3/97.

342-681 152355 Nimphius, M. J. (M.S. dissertation under direction of graduate advisor J. H. Bidanset at St. John=s University College of Pharmacy and Allied Health Professions, New York). AThe effects of chlorpyrifos and xylene on embryonal and fetal development in the rat@ (approval date: 9/13/95). Sprague-Dawley rats were dosed subcutaneously with 0, 0.3, 3, or 10 mg/kg/day chlorpyrifos (analytical grade, 99% purity) on days 1-7 of gestation (typically 8/dose/group), then sacrificed on gestation day 19 or 20. Other rats received xylene or chlorpyrifos/xylene s.c. on the same schedule. Parameters examined were resorptions, weights and lengths of fetuses, and external malformations. None of these showed biologically meaningful changes. This study is unacceptable (it does not conform to any FIFRA study design: route is not relevant to plausible human exposure, timing of dosing is not useful for evaluation of malformations, fetal examinations were only for grossly evident changes, group sizes were too small, and sacrifices were not done on a fixed gestation day). The study does not make a significant contribution to chlorpyrifos hazard assessment. Aldous, 9/3/97.

[Rat Teratology Studies: Chlorpyrifos Metabolites]

342-684 152362 Hanley, T. R., G. J. Zielke, and L. G. Lomax, A3,5,6-Trichloro-2-pyridinol: oral teratology study in Fischer 344 rats@, The Dow Chemical Co., Midland, MI, 7/23/87. Laboratory Study #: K-038278-011. Groups of 32-34 mated Fischer 344 rats were dosed with 0, 50, 100, or 150 mg/kg/day 3,5,6-trichloro-2-pyridinol (TCP, 99.7% purity) by gavage in 4 ml/kg Methocel on days 6-15 of gestation in a standard teratology study. Maternal NOEL = 50 mg/kg/day (minor body weight gain decrements). Developmental NOEL = 150 mg/kg/day (HDT). An acceptable study of a major metabolite of chlorpyrifos, with no adverse effect indicated. Aldous, 7/31/97.

TERATOGENICITY, MOUSE

**342-254 036345 Deacon, M. M., J. S. Murray, M. K. Pilny, D. A. Dittenber, T. R. Hanley, Jr., and J. A. John, "The Effects of Orally Administered Chlorpyrifos on Embryonal and Fetal Development in Mice," Dow Chemical, Toxicology Research Lab., Midland, MI, 7/24/79; Chlorpyrifos, presumed technical; 0, 0.1, 1, 10, and 25 mg/kg/day by gavage; NOEL for maternal functional toxicity = 1 mg/kg/day [cholinesterase (ChE) effects as salivation, tremors, etc.]. ChE enzyme NOEL = 0.1 mg/kg/day (significant inhibition of maternal plasma ChE at 1 mg/kg/day). Developmental toxicity NOEL = 10 mg/kg/day (decreased fetal length and weight, delayed ossification in skull, sternebrae). ACCEPTABLE, in consideration of additional information in 291:050626 (See one-liner below). Report was previously not accepted (CDFA review 2/13/86, C. Aldous). C. Aldous, 6/1/87.

291 050626 (Addendum to 254:036345, primary mouse teratology study). Dow Chemical, Midland, MI, 7/24/79. New information provides grade of test article, dates of preparation of dose solutions, individual necropsy sheets for dams dying prior to term, and rationale for selection of mouse as test animal. C. Aldous, 6/1/87.

EPA 1-liner: Teratology - mice; Toxicology. Research Lab.; 7/24/74 [sic: presumed this is the 7/24/79 study]; Teratogenic NOEL > 25 mg/kg/day (HDT); fetotoxic NOEL = 10 mg/kg fetotoxic LEL = 25 mg/kg (decreased fetal length, increased skeletal variants); Plasma and RBC ChE NOEL = 0.1 mg/kg/day.

013/053 031072 Summary of 254:036345 (see above).

342-682 152359 (Tab 43). Deacon, M. M., J. S. Murray, M. K. Pilny, K. S. Rao, D. A. Dittenber, T. R. Hanley, Jr., and J. A. John, "Embryotoxicity and Fetotoxicity of Orally Administered Chlorpyrifos in Mice@, *Toxicol. Appl. Pharmacol.* 54:31-40 (1980). This is the published report corresponding to 342-254 036345, above.

TERATOGENICITY, RABBIT

342-694 153116 Rubin, Y., A. Nyska, and T. Waner, "Pyrinex teratogenicity study in the rabbit", Life Science Research Israel Ltd., 7/15/87. Laboratory Study # MAK/103/PYR. At least 14 HY/CR (a NZW variety) rabbits per group were dosed by gavage in corn oil with chlorpyrifos (Pyrinex Technical, purity 96.1%) on days 7-19 p.c. at 0, 1, 9, 81, or 140 mg/kg/day. Maternal NOEL = 81 mg/kg/day (body weight gain decrement during treatment period). Developmental NOEL = 81 mg/kg/day [reduced crown/rump length, reduced fetal weight, ossification delays (indicated by non-ossification of fifth sternebra and/or xiphisternum)]. No adverse effects are indicated. For comparison, the pilot study had found 100% lethality in does at 270 mg/kg/day. **Acceptable. Aldous, 4/29/97.

342-685 152364 Exact duplicate of 342-694 153116, above.

[Rabbit Teratology Studies: Chlorpyrifos Metabolites]

342-684 152363 Hanley, T. R., G. J. Zielke, and L. G. Lomax, "3,5,6-Trichloro-2-pyridinol: oral teratology study in New Zealand White rabbits", The Dow Chemical Co., Midland, MI, 7/23/87. Laboratory Study #: K-038278-015. Sixteen does/group were dosed with 0, 25, 100, or 250 mg/kg/day 3,5,6-trichloro-2-pyridinol (TCP, purity 99.7%) by gavage in aqueous 0.5% Methocel on gestation days 7-19 in a teratology study. Maternal NOEL = 100 mg/kg/day (minor maternal body weight decrement during treatment). Developmental NOEL = 25 mg/kg/day (hydrocephaly and dilated cerebral ventricles). The latter observations were not statistically significantly increased in either of the two higher dose groups compared to concurrent controls, however historical background incidences were very low (compare hydrocephaly litter incidences of 2/13 and 3/13 at 100 and 250 mg/kg/day, respectively, to a historical incidence of 1/839 litters). These findings indicate a **possible adverse effect**. For perspective, 100 mg/kg/day of TCP is the molar equivalent to 66% of a chlorpyrifos dose which caused 100% mortality in LSRI Report MAK/102/PYR (cited in the accepted chlorpyrifos rabbit teratology study under DPR Record No. 153166). **Acceptable** metabolite study. Aldous, 7/31/97.

TERATOGENICITY, ANALYSIS OF ALLEGATIONS OF HUMAN DEVELOPMENTAL EFFECTS

The following critical review by Dr. J. E. Gibson and associated support documents were submitted in response to allegations that chlorpyrifos elicited human malformations.

342-680 152356 Gibson, J. E., "Critical review of allegations associating Dursban with human teratogenicity", 12/23/96 (analysis was given DowElanco Study ID JEG122396). Dr. Gibson was responding to allegations by Dr. J. Sherman that chlorpyrifos was the causative agent for several human birth defects. The most detailed version of Dr. Sherman's report was in Int. J. Occup. Med. Toxicol., 4:417-431 (1995). Dr. Gibson's primary objections to the article were (1) Dr. Sherman does not have

the training and experience to properly perform such an analysis, (2) the four cases described do not present a coherent pattern of effects, (3) the possibilities of genetic causation were ignored, even though in most cases one or more physicians experienced in evaluation of birth defects attributed findings to genetic defects (4) none of the cases offered measures of exposure, (5) statistical analysis in the article was unsound, (6) outcomes of cited animal studies were misunderstood or misrepresented, and (7) the article did not state the author=s role as paid consultant in lawsuits filed by the three affected families, which disclosure is an ethical responsibility of authorship. All lawsuits involving the four children have been dismissed. Neither the Sherman report (DPR Record No. 152349) nor Dr. Gibson=s review are primary sources of new data, hence do not have independent worksheets. **Supporting data, including some complete studies, follow in Document Nos. 342-681 to 342-686. A One-liners@ describing these submissions are found in this worksheet.** Aldous, 8/22/97.

Records submitted in support of 342-680 152356 above, included: Document No. 342-681: Record Nos. 152349, 152350, 152351, 152352, 152353 152354,152355; and Document No. 342-682: Record Nos. 152357, 152358, 152359.

GENE MUTATION

Bacteria:

255 036348 "Evaluation of Selected Pesticides As Chemical Mutagens, In Vitro and In Vivo Studies," (brief summary) SRI, 1977; Salmonella and E. coli. UNACCEPTABLE with no adverse effect reported. Salmonella, 4 strains (no TA98), were tested with and without activation at 0, 1, 5, 10, 50, 100, 500 and 1000 µg/plate and with Escherichia coli at the same concentrations. Chlorpyrifos, 98.8%. No evidence of a cytotoxic concentration or rationale for maximum concentration used. No repeat trial, no individual plate counts if more than one was made. Not upgradeable. J. Gee, 2/13/86.

273 042784 "Chlorpyrifos: Evaluation in the Ames' Salmonella/Mammalian-Microsome Mutagenicity Assay," Dow Chemical, Freeport, Texas, 1986; Salmonella. Chlorpyrifos (95.7%) tested in strains TA1535, TA1537, TA98 and TA100 at 0, 1, 3.16, 10, 31.6 and 100 µg/plate; with and without rat liver activation; 30 min preincubation before plating, triplicate plates, one trial, no evidence for increased reversion rate. UNACCEPTABLE. Report states that a precipitate formed at 100 µg/plate. The earlier study did not mention this. J. Gee, 7/30/86.

419 116728. Supplement to 042784. Contains individual plate counts and a revised table of contents. No change in the study status. No worksheet. Kellner and Gee, 7/9/93.

Mammalian cells:

255 036351 "Evaluation of Chlorpyrifos in the Chinese Hamster Ovary Cell-Hypoxanthine (Guanine) Phosphoribosyl Transferase (CHO/HGPRT) Forward Mutation Assay," Dow Chemical, Midland, MI, 1985; UNACCEPTABLE with no adverse effect reported in CHO cells. Chlorpyrifos, 95.7%, at 0, 10, 20, 25, 30, 40 or 50 uM with and without activation for 4 hours, with no increase in mutation frequency reported in a single trial. A precipitate formed at 30 uM and above. Major problem: no confirming trial. Not upgradeable. J. Gee, 2/13/86.

291 [No Record No., second "Mutagenicity" tab in volume]. Rebuttal comments ref 255:036351. CDFA conclusion was study still UNACCEPTABLE: major concern remaining is lack of a confirmatory test for a negative result. (J. Gee, 6/5/87).

291 057665 A table entitled "Analytical determination of stability of Chlorpyrifos in DMSO" in support of 255:036351, above. (Submitted as part of rebuttal document of 12/1/86).

***SUMMARY: The 1977 SRI study (#036348), using four strains of Salmonella (but not TA98) at 0 to 1000 µg/plate, was negative for increased reversion. Also, the CHO/HGPRT study on file showed negative results. EPA accepted this CHO study (#036351) although CDFA review found it unacceptable because there was no repeat. Considering all of these studies, with no one alone being acceptable, and that #042784 is a repeat of #036348 -- the deficiency for which each was rejected separately -- the 842 data gap is considered filled.

CHROMOSOME EFFECTS

** 419 116722 "Evaluation of Chlorpyrifos in an In Vitro Chromosomal Aberration Assay Utilizing Rat Lymphocytes", (Linscombe, V., Mensik D. and Clem, B., Dow Chemical Company, Lab Project Study ID: K-044793-092, 1/29/92). Chlorpyrifos, purity of 98.6%, was evaluated for clastogenic potential using rat lymphocytes treated for 4 hours with concentrations of 0 (DMSO), 5, 16.7, 50, 167.7, 500, 1667.0 or 5000 mg/ml (Assay 1) and 0, 5.0, 16.7, 50.0 and 167.0 mg/ml (Assay 2) with and without S-9 metabolic activation. Cultures were harvested 24 hours after treatment in Assay 1 and 24 and 48 hours after treatment in Assay 2. **No Adverse Effects:** No increase in chromosomal aberrations at the highest scorable dose levels of 167 mg/ml (without S-9) and 50 mg/ml (with S-9). ACCEPTABLE. (Kishiyama, Kellner and Gee, 7/1/93).

342-739 161321 Exact duplicate of 342-419 116722 (above). This was submitted in a volume which contained primarily product chemistry data. Aldous, 11/12/98.

363 087919 "Evaluation of Chlorpyrifos in the Bone Marrow Micronucleus Test." (Dow, TXT: K-044793-067A, 9/22/89). Chlorpyrifos, lot AGR 214637, 97.9%; tested with CD-1 (ICR) BR mice,

with sacrifices of 5/sex/group at 24, 48 or 72 hours after a single oral gavage dosing of 0 (corn oil) or 90 mg/kg b. wt. stated to be 80% of the LD_{50}; cyclophosphamide as positive control; no mortalities but decrease in body weights in the treatment groups; no evidence of micronuclei formation and no clear effect on PCE/NCE. UNACCEPTABLE (only one dose level). (Gee, 3/12/90)

255 036350 "Evaluation of Chlorpyrifos in the Mouse Bone Marrow Micronucleus Test," Dow Chemical, Freeport, Texas, 1985; Mouse micronucleus test. UNACCEPTABLE with no adverse effect. Chlorpyrifos, 95.7%, was given by oral gavage to 5/sex/group at 0, 7, 22, or 70 mg/kg with sacrifices at 24 and 48 hours. No statistically significant increase in micronuclei in PCE's is reported; % PCE marginally effected in females only at 48 hours being 63 as compared with 76 for the vehicle control. This is suggestive that a higher dose and/or a longer sampling time should have been included even at the risk of losing some of the animals. In the Appendix data show that survival at 100 mg/kg would be adequate for the assay. Also, no clinical signs were observed. The high dose reportedly was based on 60% of the LD50 of approximately 111 mg/kg. Guidelines and the meaningfulness of the test call for some signs than a toxic dose was reached, either the MTD for the animal or cytotoxicity to the bone marrow. The only death was in female vehicle control. No data on micronucleated normochromatic erythrocytes are included. Because positive effects have been reported in gene conversion and DNA repair, an adequate test in this test area is needed. Not upgradeable. J. Gee, 2/13/86.

NOTE: EPA considers this study as acceptable, according to the EPA response to CDFA data gap status issues on chlorpyrifos, dated 1/17/89. Aldous, 12/4/89.

291 [No Record number, first "Mutagenicity" tab in volume]. Rebuttal comments ref 255:036350. CDFA conclusion was study still UNACCEPTABLE: major concerns remaining are inadequate justification of treatment levels, and lack of a 72 hr sacrifice time. J. Gee, 6/5/87.

DNA DAMAGE

255 036349 "Evaluation of Selected Pesticides As Chemical Mutagens, In Vitro and In Vivo Studies - -Mammalian In Vitro Unscheduled DNA Synthesis Assays," SRI, 1977; UDS in WI-38. UNACCEPTABLE but upgradeable with no adverse effect reported. Chlorpyrifos, 98.8%. WI-38, human embryonic lung fibroblasts, were exposed with and without activation (rat liver) to 0, 10^{-7}, 10^{-6}, 10^{-5}, 10^{-4}, and 10^{-3} with six cultures -S9 and 3 +S9. DPM/ug DNA is reported with no change in the DPM with increasing concentrations. DNA was extracted from the cells by a standard method and an aliquot used to determine the amount of DNA and another portion used to determine the incorporation of tritiated thymidine by liquid scintillation counting as a measure of DNA repair in response to damage by the test article. Missing information on how the CPM were converted to DPM, the quantity of DNA recovered per culture, the passage number of the WI-38, and the rationale for the selection of the concentrations used - whether solubility or cytotoxicity. CDFA review 2-13-86 J. Gee.

255 036347 "Evaluation of Selected Pesticides As Chemical Mutagens, In Vitro and In Vivo Studies - -Microbiological Assays" (summary report), SRI, 1977; Saccharomyces cerevisiae D_3. UNACCEPTABLE with a positive effect reported. Mitotic recombination-gene conversion in yeast exposed to a 5% concentration for 4 hours, with and without metabolic activation. The test was repeated. No individual data. Because of the lack of data, the significance of the effect cannot be evaluated but the possible genotoxic effect must be noted. Upgradeable. J. Gee, 2/13/86.

255 042609 "Evaluation of Selected Pesticides As Chemical Mutagens, In Vitro and In Vivo Studies - Microbiological Assays" (summary), SRI, 1977; Escherichia coli and Bacillus subtilis. UNACCEPTABLE with a positive adverse effect reported. Chlorpyrifos, 98.8% purity, at 2.5 µg/disc, was tested with E. coli W3110 and p3478 and with B. subtilis H17 and M45. No activation was included and the test reportedly was repeated 3 times. The comparable zones of inhibition between the strains indicated a larger zone for the repair defective strains. Only one value for each strain is reported. If the full report were submitted, it is possible that the effect could be evaluated for significance. Since no activation was included, the study is not upgradeable. J. Gee, 2/13/86.

** 273 042785 "Evaluation of Chlorpyrifos in the Rat Hepatocyte Unscheduled DNA Synthesis (UDS) Assay," Dow Chemical, Midland, MI, 1986; Chlorpyrifos (95.7%); primary rat hepatocytes tested for unscheduled DNA synthesis at 10^{-6}, 3.13 x10^{-6}, x 10^{-5}, 3.16 x 10^{-5} and 1 x 10^{-4} M; triplicate cultures in a single trial; no evidence of UDS; toxicity at the highest concentration. Acceptable. J. Gee, 7/30/86.

SUMMARY: The positive findings in the two microbial studies are somewhat related. The B. subtilis test compares the response of rec$^-$ (recombination defective) with wild type organisms. The rec$^-$ strain is not as competent to repair damage and hence shows a greater inhibition of growth from lethality due to DNA damage. The test in Saccharomyces also measures recombination-type events in competent organisms and the increase in these events confirms the DNA damage. The complete versions of these two reports are needed to assess their significance. The two tests in mammalian cells measure a different repair event (excision repair) with repair replication occurring to fill the DNA gap following removal of damaged bases by excision using different enzymes. Although the data gap for 844 is filled, the positive findings in the microbial tests cannot be dismissed without more information about the bacterial studies.

NEUROTOXICITY

Hen

**291 051119 "Chlorpyrifos: Subchronic Organophosphate-Induced Delayed-Neurotoxicity (OPIDN) Study In Laying Chicken Hens," (Report No. TXT:K-044793-064), Health & Environmental Sciences, Dow Chemical, Freeport, Texas, 4/86. Chlorpyrifos, tech. (approx. 96% purity). 0, 1, 5, and 10 mg/kg/day. No evidence of delayed distal neuropathy. 10 mg/kg/day chlorpyrifos caused

weight loss, diminished egg laying capacity, and transient abnormal gait (fully reversible between dosing periods, and not persistent throughout study). Study fills neurotoxicity data requirement. C. Aldous, 6/3/87.

255 036346 "Acute Delayed Neurotoxicologic Evaluation of Chlorpyrifos in White Leghorn Hens," Dow Chemical, Lake Jackson, Texas, 5/22/78; Chlorpyrifos, tech; 0, 50, and 100 mg/kg (gelatin capsule); NOEL = 100 mg/kg for behavioral or microscopically evident delayed neuropathy (Highest dose tested) NOT ACCEPTABLE, not complete, not upgradeable (no repeat dosage at day 21 when no effects were observed, not all currently required tissues examined.) C. Aldous, 2/13/86.

EPA 1-liner: [Acute delayed neurotoxicity - hen; Dow; 5/22/78] LD50 in hens= 50 mg/kg Negative @ 50 & 100 mg/kg. Core grade, minimum.

342-496 132855 ADowElanco chlorpyrifos joint neurotoxic action of chlorpyrifos and safrotin in hens (Duke Univ. Medical Center Dept. of Physiology and Pharmacology, Durham, NC). Assigned to Worker Health and Safety Branch for review. (Aldous, 8/8/97).

342-745 162520 (No Author) APreliminary Report: Assessment of neurotoxicity associated with co-exposure to the organophosphorus insecticides chlorpyrifos and diazinon@. White leghorn hens were dosed with maximal levels of chlorpyrifos and/or diazinon and kept alive with atropine and 2-PAM for 96 hours prior to sacrifice and assays of ChE (plasma and brain), and brain NTE. There were apparently cumulative effects for brain and plasma ChE. Although diazinon by itself did not affect NTE activity, diazinon potentiated the NTE inhibition of chlorpyrifos from 35% to 65% of normal. There is insufficient information in this preliminary report to warrant a Medical Toxicology Branch worksheet. Aldous, 11/09/98.

Rat (includes a variety of related study types in this species)

342-445 126304, "Chlorpyrifos: 13-Week Neurotoxicity Study in Fischer Rats", (Shankar, M., Bond, D. and Crissman, J., Dow Chemical Company, Laboratory Project K-044793-094, 9/16/93). Chlorpyrifos, purity 98.1%, was administered in the feed at concentrations of 0, 0.1, 1, 5 or 15 mg/kg to 10 Fischer 344 rats/sex/group for 13 weeks. High-dose males and females had reduced motor activity at week 4. Perineal soiling (low incidence) was observed for 5 and 15 mg/kg/day groups; NOEL (for clinical signs, FOB, motor activity) = 1 mg/kg/day. No histopathologic findings. Neuropathologic NOEL = 15 mg/kg/day. **No Adverse Effects. Report was originally classified as unacceptable, but upgradeable. Data provided in Record No. 132458 (see below) allowed an upgrade to **acceptable** status. This study type is considered "supplemental" under SB 950 at this time. Kishiyama, Kellner and Gee, 7/6/94; Aldous, 4/8/97.

342-493 132458 (Addendum to Document # 342-445, Record # 126304). Cover letter dated 10/4/94. The three primary acceptability concerns expressed in the original DPR review have been adequately addressed: characterization of technical and treated diets for content, stability, and homogeneity; ChE inhibition data as evidence that selected dose levels were appropriate; and evaluation of statistical significance for major parameters of this study. Data obtained from a 1988 subchronic feeding study found ChE enzyme inhibition NOEL = 0.1 mg/kg/day (inhibition of plasma ChE in both sexes and of RBC ChE in females at 1 mg/kg/day). ChE-related clinical effects NOEL = 1 mg/kg/day (perineal staining in occasional females at 5 and 15 mg/kg/day). Motor activity reduction, at 15 mg/kg/day during the week 4 evaluation only, was confirmed statistically. NOEL for findings other then probable acute ChE effects = 15 mg/kg/day (HDT). The study is re-classified as **acceptable**, with **no adverse effects** other than expected ChE inhibition and associated changes. Aldous, 4/8/97.

342-448 126408 Wilmer, J., et. al. "Chlorpyrifos: Acute Neurotoxicity Study in Fischer 344 Rats", (Dow Chemical Company, Study ID: K-044793-093B, 9/11/92). Chlorpyrifos (purity 98.1%, lot #MM-890115-616) was administered in a single oral gavage to 10 Fischer 344 rats/sex/group at levels of 0, 10, 50 or 100 mg/kg. Body weights of mid- and high-dose rats were significantly reduced on day 2 but not on day 8 or 15. Clinical signs (increased perineal soiling) in mid- and high-dose rats and FOB observations (incoordination, decreased muscle tone, tremor, increased lacrimation and salivation) in high-dose females were seen soon after dosing (day 1). Motor activity was reduced in mid- and high dose rats on day 1; some reductions persisted to day 8 in high-dose females. NOEL (Body wt., Clinical signs, FOB and motor activity) = 10 mg/kg. No histopathologic changes. NOEL (histopathology) = 100 mg/kg. **No Adverse Effects. Original DPR review had requested additional purity, stability and homogeneity data on the dosing material, justification for dose level selection, and clarification of the statistical methods used, as criteria for Aacceptable@ status. These data were provided (see review for Record No. 132457, below) and report is now **acceptable**. This study type is classified as "supplemental" for SB 950 at this time. Kellner and Gee, 7/5/94; Aldous, 4/9/97.

342-492 132457 [Cover letter referencing supplementary data was by Blewett, T. C. The acute range-finding study in this record supporting dose selection for the acute neurotoxicity study was by Wilmer, J. W. *et al.* (Study ID K-044793-093A)]. Addendum to Document # 342-448, Record # 126408 (rat acute neurotoxicity). Cover letter date: 10/4/94. The three primary acceptability concerns expressed in the original DPR review have been adequately addressed: characterization of technical and treated diets for content, stability, and homogeneity; rangefinding study clinical signs data as evidence that selected dose levels were appropriate; and evaluation of statistical significance for major parameters of this study. In the range-finding study, two F344 rats/sex/group were dosed once by corn oil gavage at 50, 100, 150, and 200 mg/kg. Clinical signs consistent with ChE inhibition peaked at about 6 hr after dosing. Major signs were decreased activity, incoordination, lacrimation, muscle twitches, perineal soiling, salivation, and tremors. These signs were well established at 100 mg/kg and above, especially in females. Rangefinding study data are sufficient to justify dose levels used in the neurotoxicity study. Additional statistical data are consistent with interpretations in the original DPR review. The study is re-

classified as **acceptable**, with **no adverse effects** other than expected ChE inhibition-associated changes. Aldous, 4/9/97.

448 126409 Spencer, P. et. al. "Positive Control Exercises: Motor Activity, Functional Observational Battery and Neuropathology". Dow Chemical Co. submitted this report in support of -445:126304 and -448:126408; it contains validation studies of motor activity tests, functional observational battery (FOB) assays and neuropathological examinations using rats that were administered compounds with well-documented neurotoxic potential. This document was found to be ACCEPTABLE to satisfy the FIFRA guidelines for positive controls. An evaluation of these studies is included in the background sections of the acute and 13-week rat neurotoxicity studies mentioned above. No Worksheet. Kellner and Gee, 7/18/94.

342-747 162522 Maurissen, J. P., M. R. Shankar, and J. L. Mattsson, AChlorpyrifos: cognitive study in adult Long-Evans rats@, The Dow Chemical Co., Midland, MI, 4/29/96, Laboratory Project ID: K-044793-096. Female Long-Evans rats were dosed by gavage in corn oil with 0, 1, 3, or 10 mg/kg/day chlorpyrifos (98.1% purity) for 4 weeks. The cognitive study was a Adelayed matching to position task@ design. Cognitive testing was done during each of the treatment weeks and for 4 weeks thereafter, by methods described below. Rats were placed on modest food restriction to provide incentive to seek the Afood reward@ in the study. Rats were trained and selected for the study, based on positional memory performance. In a given test, a rat was presented with one of two retractable levers. The rat was to press the lever offered, cross the cage and interrupt a beam at the food cup within 10 seconds, and then return to the side of the cage with the levers. At this time, both levers would be presented. The rat was expected to select and press the correct lever (i.e., the one just presented a few seconds earlier) within 10 seconds after leaving the food cup station. A correct choice made a food reward available at the food cup. In addition to the above test, the task was made more difficult by involving progressively longer delays (up to 15 seconds) between the first lever press and the time in which a nosepoke in the food cup would extend the levers (called the delayed matching-to-position or ADMPT@ paradigm). These rats were also examined twice daily on treatment days during the 4-wk dosing period: observations were about 3 hr and 21 hr after the most recent treatment. Satellite groups of 6/dose/interval were used for ChE assays and brain NTE assays on the day following the last treatment, and 1 month after the last treatment. The 1998 DPR review placed the NOEL for memory retention at 3 mg/kg/day (considering a small apparent memory retention change at 10 mg/kg/day to be a Apossible adverse effect@). **This determination was subsequently changed (see review for Document No. 342-789, immediately below). NOEL for clinical observations is 1 mg/kg/day (miosis). There is no NOEL for ChE inhibition (marked inhibition of plasma and RBC ChE and modest (8%) inhibition of brain ChE at 1 mg/kg/day). Some high dose observations associated with the DMPT tests were appropriately considered by investigators to have been attributable to motor slowing and/or decreased motivation (increased Aactual total delay@, increased Avoid trials@, and decreased numbers of nosepokes per trial). None of these were noted after the end of the treatment period. Report was originally classified as not acceptable (requiring dosing solution analysis). Such data were subsequently provided (see immediately below). Study is **acceptable**. Aldous, 11/6/98, 10/12/99.

342-789 168961, 168962, and 168963. **Supplemental information to the above cognitive study (Record 342-747 162522)**. Additional data and explanatory text were provided. Essential responses summarized below are detailed in review AW162522 s01.wpd@. New data supplied dosing solution analyses, and additional tables showing mean correct responses for individual animals and for treatment groups, including methodology used to obtain memory retention slope values. **These data allow an upgrade of Record No. 162522 to acceptable status.** In addition, investigators provided a statistical analysis of slopes of the memory retention curves for the various treatment groups. Data show that there were no significant responses, hence data **do not demonstrate a possible adverse effect** (a change from the previous review). The variability of the data is sufficiently large that only a very substantial decrease of memory retention would have been detectable, thus the present study conditions **did not provide a sensitive test**. Aldous, 10/12/99.

Rat (Developmental Neurotoxicity)

**342-746 162521, Hoberman, A. M., ADevelopmental neurotoxicity study of chlorpyrifos administered orally via gavage to Crl:CD7(SD)BR VAF/Plus7 presumed pregnant rats@, Argus Research Laboratories, Inc., 5/1/98. Sponsor Protocol No. K-044793-109; Argus Study ID 304-001. Crl:CD7(SD)BR VAF/Plus7 presumed pregnant rats were gavaged on gestation day 6 through lactation day 11 with chlorpyrifos (99.8%) in corn oil at 0, 0.3, 1, and 5 mg/kg/day. Initially there were 25 dams/group on treatment. On lactation day 5, twenty litters/treatment were continued on study. Four subsets of 20 pups/sex/group were selected on lactation day 5, each consisting of 1/sex/litter. Primary investigations for the subsets were: (Subset 1): morphometric evaluations and histopathology of brains after postpartum day 12 sacrifice, (Subset 2): spatial delayed alternation studies at postpartum days 23-25 and 62-91, (Subset 3): motor activity testing on postpartum days 14, 18, 22, and 61: auditory startle on postpartum days 23 and 62, (Subset 4): evaluation of developmental landmarks (pinna unfolding, eye opening, preputial separation or vaginal opening); brain weight evaluation in 10/sex/group sacrificed during lactation days 66-71, and neurohistopathology following *in situ* perfusion of 6/sex/litter. Maternal NOEL = 0.3 mg/kg/day (brain ChE inhibition). Clinical signs of ChE inhibition were observed in 5 mg/kg/day dams. Developmental NOEL = 1 mg/kg/day (decreased neonatal survival; decreased pup growth, with 11% reduction in body weight at 66 days postpartum in males; maturational delays of pinna unfolding, preputial separation in males, and vaginal patency in females; reduced morphometric dimensions of cerebellum and hippocampal gyrus at day 12 postpartum compared to concurrent and historical controls, reduced morphometric dimensions of parietal cortex and hippocampal gyrus at day 66 postpartum compared to concurrent and historical controls in high dose females, reduced motor activity at day 14 postpartum, reduced auditory startle habituation peak response and increased latency to response at day 23 postpartum). This study was classified as Anot acceptable but upgradeable@ in the initial review, with the primary concern being appropriateness of the validation studies for evaluation of spatial delayed alternation. The response in Record No. 168955 (below) addressed the advantages of the using memory retention as a function of time for validation of technique, as compared with memory

reduction due to exogenous chemicals. The investigators= response gave examples of many confounding effects of exogenous chemicals on parameters other than on memory. Study findings are not of sufficient magnitude or persistence to be considered as Aadverse@. Report is now **acceptable**. Aldous, 11/13/98 and 9/17/99.

342-769 164347 Submission of morphometry and histopathology data on F1 rats sacrificed after day 66 in Record No. 162521, above. Data were incorporated into the review for the main study under that Record Number. Aldous, 11/12/98.

342-789 168955, 168959, and 168960. Supplemental information to developmental neurotoxicity study 342-746 162521. Final report date of update: 5/7/99. Additional data and explanatory text were provided, **allowing an upgrade of Record No. 162521 to acceptable status**. Essential responses summarized below are detailed in review As162521 s01.wpd@. The validation studies for evaluation of spatial delayed alternation, which were based on temporal patterns of memory performance over sufficient duration to show a consistent linear change over time, were shown to be satisfactory. Representative micrographs prepared by the pathologist were presented, demonstrating several of the commonly encountered lesions following insult to the several areas of the CNS, dorsal root ganglia, and peripheral nerves. Additional brain morphometric data requested by U.S. EPA were provided, plus selected published articles. One article showed that undernutrition reduces pup brain weight increases, although to a much lesser extent than the decrement of body weight gain. Another article determined that the reductions of dimensions in brain regions appear to affect all brain morphometric measurements proportionately. A third article showed that undernutrition leads to locomotion delays which are quite remarkable during lactation days 14-16, whereas some components of coordinated movement and altered posture remain affected for a longer time. Aldous, 9/17/99.

342-832 (suppl. to 342-746) 182481 (suppl. to 162521) Hoberman, A. M., Report Supplement 3 to: ADevelopmental neurotoxicity study of chlorpyrifos administered orally via gavage to Crl:CD7(SD)BR VAF/Plus7 presumed pregnant rats,@Argus Research Laboratories, Inc., dated 5/1/98 (of original study), this supplement dated Oct. 9, 2000. Protocol No. of this supplement: 304-001. Brain morphometric data from the original report were re-tabulated alongside historical control data from 4 or 5 studies per parameter. Only one measurement having a high dose value statistically significantly different from concurrent controls was outside the range of the historical controls: the cerebellar anterior/posterior dimension in 5 mg/kg/day male 12-day pups was significantly below concurrent control dimension, and also outside the range of the available historical controls. Females did not suggest such a relationship at 12 days, and neither sex showed altered cerebellar anterior/posterior distance after 66 days. In the context of the demonstrated high maternal and neonatal toxicity of this dose, the supplemental data reinforce the lack of demonstrated special toxicity of the test article toward the developing nervous system. Supplemental to a previously acceptable study with no adverse effects. Aldous, 9/26/01.

342-824 178362 [Same report as 342-746 162521, above].

Human Epidemiological Studies Related to Neurotoxicity

(This is not an exhaustive list, since primary responsibility to evaluate these studies belongs to Worker Health and Safety Branch)

342-543 138174 Nolan, R. J. (Study Director) ACritical analysis of the allegations of neuropathy due to chlorpyrifos submitted to the United States Environmental Protection Agency on November 7, 1994". DowElanco had identified 31 individuals for whom physicians had made at least tentative diagnoses of neuropathy having possible association with chlorpyrifos. Although several cases of massive chlorpyrifos exposure had previously been documented, only one appeared to have caused organophosphate-type delayed neuropathy (OPIDN): this was an attempted suicide in which heroic treatments were required to address severe cholinergic symptoms (investigators citing Lotti *et al.*, 1986). The primary focus of the present investigation was on OPIDN symptoms, however other neurological findings were noted where found. None of the exposures (or worst plausible estimates of exposures) were judged to have been Abiologically significant@ [i.e., exposures were likely to have been too low to have measurably depressed plasma ChE, or (for inhalation route) were less than the NAS guideline of 10 µg/m^3]. Studies to date have indicated that it is critical to achieve at least 50% inhibition of neurotoxic esterase in order obtain OPIDN symptoms: this is unlikely to happen except at dose sufficient to elicit major cholinergic crises. Onsets of acute symptoms in this study were compared with plausible response times for acute ChE inhibitory signs (usually within 4 hr, in any case within 24 hr). The majority of cases presented no cholinergic signs, and none presented signs which were unambiguously due to ChE inhibition. Only three persons had documented neuropathy which became evident within one month of alleged exposure (a plausible time frame for OPIDN), without a demonstrated alternate cause. Of these, no two of them had consistent symptoms. DowElanco therefore determined that the alleged neuropathologies could not reasonably be attributed to chlorpyrifos. No SB-950 worksheet is appropriate, since this is not a relevant study type, and data do not support a treatment effect. Aldous, 8/11/97.

342-707 154147 ACritical assessment of reported entitled >Review of chlorpyrifos poisoning data=A. This report was directed to Worker Health and Safety Branch for review, since the commonly expected poisoning incidents would be acute cholinergic events. No Medical Toxicology Branch review has been requested. Aldous, 8/11/97.

MISCELLANEOUS [includes metabolic disposition and cholinesterase (ChE) inhibition]

342-790 168952 Chen, W. L., R. J. Nolan, and J. L. Mattsson, "Dow AgroSciences' response to the report of the Hazard Identification Assessment Review Committee (HIARC) entitled 'Chlorpyrifos - Hazard Identification Based on Animal Studies'". This record was an evaluation of existing data, and not a report of new data, except for an abstract of a recent human study by Kisicki *et al*. (reviewed as DPR Record No. 168932, see 1-liner below). "Laboratory Study ID's # GH-C 4904. This record was provided to call to question key U.S. EPA conclusions regarding hazard evaluation of chlorpyrifos. **Human clinical sign evaluation:** The cited abstract concluded that the NOEL for RBC AChE was 1 mg/kg, based on 1/12 volunteers having over a 17% decrease in this enzyme at 2 mg/kg. None of the 12 volunteers at the highest dose of 2 mg/kg experienced clinical symptoms. This result suggest that a single subject presenting signs of "blurred vision, feeling of faintness, and runny nose's in an earlier study at 0.1 mg/kg/day was unlikely to have been responding to chlorpyrifos treatment. **Relevance of RBC AChE vs. BuChE:** Registrants observed that the latter has no known physiological function and no apparent relevance to human hazard assessment. In contrast, RBC AChE is evidently identical to the AChE associated with neuromuscular transmission, hence relevant in human hazard assessment. **Comparative inhibition of AChE from different sources:** Rat studies over the dose range of 10 to 100 mg/kg indicated that RBC AChE had a 12-fold lower ED_{50} than whole brain, hence regulation on blood AChE would protect against cholinergic toxicity. AChE in other tissues was less sensitive to inhibition (i.e. had a higher ED_{50}) than whole brain (p. 22). **Primary conclusions of investigators:** Investigators determined (1) that human data are valid and preferable to animal data in assessing human hazard, (2) that human RBC AChE rather than BuChE should be used to set RfD's, (3) and that the laboratory animal data base (if agencies are determined to use such for human safety assessment) is sufficiently complete that (a) there is no justification for an additional ten-fold safety factor for uncertainties regarding possible special toxicity to infants and children and (b) the comparative blood ChE responses of humans and laboratory animals (for RBC AChE and BuChE) are sufficiently well-characterized that a 10-fold interspecies uncertainty factor is not appropriate. Supportive published articles were included: (1) Chen *et al*. "Human red blood cell acetylcholinesterase inhibition as the appropriate and conservative surrogate endpoint for establishing chlorpyrifos reference dose', **Regulatory Toxicology and Pharmacology 29**, 15-22 (1999), (2) Schardein and Scialli, "The legislation of toxicologic safety factors: The Food Quality Protection Act with chlorpyrifos as a test case', **Reproductive Toxicology 13**, 1-14, 1999, and (3) Gibson, J. E. *et al.*, "How to determine if an additional 10x safety factor is needed for chemicals: A test case with chlorpyrifos', **Toxicological Sciences 48**, 117-122 (1999). No worksheet (no reviewable data). Aldous, 9/14/99.

342-788; 168932; " A Rising Dose Toxicology Study to Determine the No-Observable-Effect- Levels (NOEL) for erythrocyte Acetylcholinesterase (AChE) Inhibition and Cholinergic Signs and Symptoms of Chlorpyrifos at Three Dose Levels'; (Kisicki, J.C. *et. al.*; MDS Harris, Lincoln, Nebraska; Study ID. DR K-044793-284; 4/19/99); Six male and six female human volunteers/treatment group were fasted overnight prior to being dosed orally once with 0 (placebo: lactose monohydrate), 0.5 or 1.0 mg/kg of

chlorpyrifos powder (purity: 99.8%) in capsules (phase 1) or 0 or 2.0 mg/kg (phase 2) in a double blind, randomized study. The health status of each subject was monitored for up to 7 days. Vital signs (blood pressure, pulse rate, respiration rate, and body temperature) were recorded prior to dosing and at 1, 2, 4, 8, 12, 24, 48 and 168 hours after dosing. Blood samples for erythrocyte acetylcholinesterase (AChE) analysis were drawn 10 hours prior to dosing, at the time of dosing and at 2, 4, 8, 12, 24, 36, 48, 72, 96, 120, 144 and 168 hours post-dose for erythrocyte AChE activity and chlorpyrifos and metabolite analyses. A blood sample was drawn prior to dosing for paraoxonase activity determination. Urine samples were collected at 12 hour intervals starting 48 hours prior to dosing and at 0 to 6 and 6 to 12 hours post-dose and 12 hour intervals thereafter up to 168 hours after dosing. Although clinical symptoms such as anorexia, diarrhea, nausea, vomiting, dizziness, dyspnea, and headache were reported, none of these signs occurred in a dose-related manner. There was no apparent treatment-related effect upon any of the vital signs. Mean erythrocyte AChE activities were not significantly affected in a dose-related manner. One subject in the 2.0 mg/kg treatment group demonstrated a maximal 30% inhibition between AChE activity reported at 0 time and at 12 hours post-dose. Otherwise, no other subject in the high dose group had a reduction in erythrocyte AChE activity greater than 12% based on the higher of the two baseline values. The blood and urine levels of chlorpyrifos and its metabolites and the paraoxonase activity analysis for individual subjects were not included in this initial report and thus could not be evaluated. **No adverse effects indicated. NOEL:** 1.0 mg/kg (based upon the 30% inhibition of erythrocyte AChE demonstrated by one of the subjects in the 2.0 mg/kg treatment group). **Supplemental Study.** (Moore, 5/18/99).

342-823 178361 This is a copy of study 342-788; 168932, above.

342-822 178360; Acetylcholinesterase (AChE) Inhibition Study; Human; The Dow Chemical Company, Midland, MI; Laboratory I.D. No. 981176; 6/5/00; Chlorpyrifos; Human volunteers (6/sex/dose) received a single oral dose of 0.0, 0.5, 1.0 or 2.0 mg/kg (capsule form) in a double-blind clinical trial; blood and urine specimens were collected and analyzed for chlorpyrifos and its metabolites (chlorpyrifos oxon and 3,5,6-trichloro-2-pyridinol (TCP)) using GC-MS; pretreatment Chlorpyrifos Oxonase (CPOase), paraoxonase and diazoxonase were determined spectrophotometrically; blood and urine specimens were generally below the limit of quantitation (LOQ) for chlorpyrifos; average AUC for TCP in blood (by increasing dose) was 14.0, 25.2 and 51.2 µg/g, respectively and amount TCP excreted in the urine was 4.1, 8.7 and 15.9 mg, respectively during the first 168 hr following ingestion; blood and urinary TCP levels increased rapidly, remained constant over first 48 hr post-treatment, and then declined with an average half-life of 29 to 36 hr; administration by capsule probably reduced absorption (average of 34.7%, 30.8% and 29.5% absorbed in 0.5, 1.0 or 2.0 mg/kg dose group, respectively); serum CPOase activity was within the range of activity reported in previous studies and there were no extreme values; RBC ChE depression was seen in only one individual, a 2.0 mg/kg female that showed unusually high absorption of chlorpyrifos (87.9% versus 29.5%). Supplementary Data. Kellner, 2/23/01. [NOTE by C. Aldous: This study is APart B@ of 342-788; 168932, above].

342-763 164102 Mendrala, A. L. and K. A. Brzak, "Chlorpyrifos: Part A - Concentration - time course of chlorpyrifos and chlorpyrifos-oxon in blood", The Dow Chemical Co., Midland, 8/31/98, Laboratory Project Study ID 971187A. Chlorpyrifos was administered by gavage in corn oil to male F344 rats at dose levels of 0.5 to 100 mg/kg. [Segment 1]: Four rats/group were killed at intervals of 10 min to 12 hr to determine time course of (a) concentrations of chlorpyrifos and chlorpyrifos-oxon, and (b) plasma and brain cholinesterase (ChE) activities. Chlorpyrifos concentrations peaked at 3 hr, with levels dropping substantially at 6 to 12 hr. Chlorpyrifos-oxon was only about 1% as abundant as chlorpyrifos, and was typically detectable at 1 hr and 3 hr intervals only. Plasma ChE inhibition was evident at all dose levels (15% inhibition at 0.5 mg/kg). Brain ChE was marginally evident at 5 mg/kg (NOEL = 1 mg/kg). [Segment 2]: Four rats/group were dosed by gavage in corn oil with nominal 5 or 100 mg/kg (achieved levels of 3 and 63 mg/kg) of ring-labeled ^{14}C-chlorpyrifos 3 hr prior to sacrifice. Blood was collected for measurements of circulating chlorpyrifos, chlorpyrifos-oxon, and the trichloropyridinol (TCP) hydrolysis product. TCP was by far the most abundant labeled species found in blood (about 98% of label at either dose level), with most of the remaining label as chlorpyrifos. Useful supplemental data, no DPR worksheet. Aldous, 10/13/99.

342-763 164103 "Effects of chlorpyrifos administered via gavage to CD rats during gestation and lactation on plasma, erythrocyte, heart and brain cholinesterase, and analytical determination of chlorpyrifos and metabolites", The Dow Chemical Co., Midland, 08/98. This study was not reviewed under SB-950, but has been examined extensively by R. Cochran for the chlorpyrifos risk assessment. Aldous 10/13/99.

342-763 165484 Griffin, P., H. Mason, K. Heywood, and J. Crocker, "Oral and dermal absorption of chlorpyrifos: A human volunteer study", cover letter dated 11/23/98. (This was a manuscript accepted for publication in Occupational & Environmental Medicine). Data were reviewed by T. Thongsinthusak of DPR Worker Health and Safety Branch: that review is bound with the volume. Dermal applications led to 1% absorption (evidenced as dialkylphosphate urinary metabolites), and 53% unaltered chlorpyrifos was recovered by washing the application site. Investigators did not account for the balance for the remainder of residues. Aldous, 10/13/99.

342-774 165918 "Standard operating protocol for analysis of the effects of chlorpyrifos, diazinon, and sulfotepp on neurite length in differentiating neuroblastoma cells in vitro." This volume is currently in evaluation by another division of DPR, and appears unlikely to be pivotal to Medical Toxicology Branch, based on its title. There are, however, studies in the public literature relating to chlorpyrifos effects on differentiating cells in culture, hence this protocol may be supportive of such a study. C. Aldous, 10/13/99.

342-756 162540 Albers, J. W. *et al.*, "Determination of the reference dose for chlorpyrifos: Expert panel report." No date was given for report: cover letter date for volume was 6/19/98. Dow Agrosciences convened a panel of experts, who determined in this 85-page record that (1) multiple studies support an RfD for repeated oral dose exposure of 0.01 mg/kg/day, and

(2) the RfD for single oral exposure was determined to be 0.05 mg/kg. There are no new studies, hence no DPR worksheet. Aldous, 10/13/99.

342-833 182482 Baker, P. C. *et al.*, ACommunication: Preliminary evaluation of acetylcholinesterase (AChE) in brain, peripheral tissues, and RBC in beagle dogs,@ The Dow Chemical Company, Midland, MI, 5/11/01. Report ID CPF0501. [Report begins on p. 38 of this volume]. Three males/group were dosed in diet with 0, 0.3, 0.6, or 1.2 mg/kg/day chlorpyrifos for 28 days. Parameters evaluated at termination focused on acetylcholinesterase measurements in RBC=s, brain, nodose ganglion, left atrium, left ventricle, diaphragm muscle, and thigh muscle. In-life RBC acetylcholinesterase activity was measured weekly. All dogs survived the treatment, and there were no characteristic clinical signs. Body weight was unaffected by treatment. RBC acetylcholinesterase activity was reduced in dose-related fashion. Despite high variability in control activities, reductions in the higher two dose levels were clearly treatment-related (about 50% reduction at 1.2 mg/kg/day). These changes appeared to be progressive over time. No other tissues showed statistically significant reductions in AChE activity. Some of the assayed AChE activity values were so variable that the small numbers of dogs available could only have indicated major treatment responses. This is a useful pilot study, but data are unsuitable for quantitative analysis. Aldous, 9/27/01.